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Interfacial Photoreactions and Chemical Capacitance in Lipid Bilayers

(tunable voltage clamp/membranes/porphyrin/laser)

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ABSTRACT **The electrical response of a pigmented lipid bilayer to a short laser pulse is measured by a *tunable voltage clamp method*. In this method, a variable access impedance permits "tuning" of the observed relaxations for optimal measurements. Analysis of the data so obtained leads to an equivalent circuit that contains a novel *chemical capacitance* charged by the specific photoreaction across a single membrane–water interface. This chemical capacitance is distinct from the ordinary membrane capacitance. The intrinsic chemical rate constant obtained from the equivalent circuit analysis is shown to be the pseudo-first-order rate constant of the reverse dark reaction of the reduced acceptor and the oxidized pigment. The tunable voltage clamp method of measurement and analysis allows unambiguous separation of this rate constant into resistive and capacitative elements, which are interpreted in molecular terms.**

Bimolecular lipid membranes (lipid bilayers) which contain pigments are excellent models of photobiological systems. Some previous studies have used continuous light excitation (1–5), and it is difficult to deduce a mechanism from steady state measurements alone. Excitation with pulsed light allows kinetics to be determined, but most previous studies have been limited either in the instrumental time resolution or in the analysis of the system responses (6–12). The frequently used open circuit voltage measurement always includes the large membrane RC (resistance–capacitance) time constant in its relaxations. This time can be reduced by shunting the membrane resistance but only at the expense of a decreased voltage amplitude. The voltage clamp method (13) wherein a fast negative feedback amplifier maintains a fixed voltage across its input is the apparently ideal solution to this problem. This method is usually limited by the access impedance of the electrodes and the electrolyte solutions, and it is difficult to achieve a true voltage clamp at the membrane–water interfaces (ideal voltage clamp) with an instrumental time constant of less than 10 μsec. Besides, one is dealing in general with a time-variant photoemf. The assignment of an observed photoelectrical signal as an emf change and/or a conductance change is not always straightforward even in the steady-state measurements (5). Our analysis shows that the ideal voltage clamp measurement provides only one time constant, that of the chemical reaction. If, however, the access impedance is included in the circuit analysis, a more complete measurement and description of the properties of the membrane photosystem is possible, and yet the deviation from ideality in terms of error voltage is negligible. We call this method a *tunable voltage clamp method*, since the time constants and the gain of the system can be optimized to measure the membrane parameters by varying the access impedance.

We shall illustrate this method with results obtained from a simple photosensitive membrane system excited by a short laser pulse. It consists of a lipid bilayer which contains a magnesium porphyrin and separates two aqueous solutions of oxidant and reductant. The electrical response to short pulsed light is specific to the thin bimolecular region of the membrane and can be made specific to a single membrane–water interface (14). Analysis of the results leads to an equivalent circuit which contains a novel capacitance charged by the specific oxidation-reduction (redox) reaction across the interface. This capacitance is clearly distinct from the ordinary membrane capacitance, and we name it the *chemical capacitance* (C_p in Fig. 1). The present method allows the separation of the intrinsic chemical rate constant ($1/\tau_p$ in Fig. 1) into resistive and capacitative elements, which are interpreted in molecular terms.

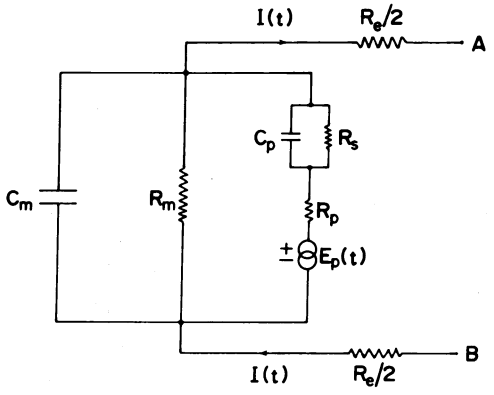
MATERIALS AND METHODS

Methods of forming the pigmented membrane are described elsewhere (5, 14). The underlying chemical reactions in the membrane–water system are shown in Fig. 2. Usually, the aqueous phases are made asymmetrical so that one aqueous phase contains predominantly oxidant (oxidant side), while the other aqueous phase contains either no redox reagents or predominantly reductant (reductant side). The tunable voltage clamp circuit used in this study consists of a fast operational amplifier in inverted mode with variable access impedance at the input (see *legends* of Figs. 1 and 3.). The output of this amplifier is connected to a signal averager and is eventually read out on an XY-recorder. The light source is a rhodamine 6G dye laser pulse with 0.3 μsec half-width at 590 nm.

RESULTS AND DISCUSSION

The electrical relaxation of the membrane system after excitation by a very short pulse of light is biphasic (Fig. 3). The relaxation can be fitted with two exponential functions of opposite polarity; the ratio of the amplitudes closely equals the reciprocal of the ratio of the respective time constants. The relaxation times can be made smaller by progressively adding a small amount of ferrocyanide to the oxidant side of the aqueous phases. Relaxation times of the positive component as fast as 5 μsec have been recorded.

To account for these observations, we propose the equivalent circuit shown in Fig. 1. Comparison with our previously proposed equivalent circuit for the continuous light responses (Fig. 1*A* of ref. 5) shows that a parallel combination of R_s and C_p is added in series to the seat of photoemf



$$I(t) = \frac{1}{R_e C_m R_p\left(\frac{1}{\tau_s} - \frac{1}{\tau_l}\right)}\left[\left(\frac{1}{\tau_s} - \frac{1}{R_s C_p}\right)\int_0^t E_p(u)\exp\left(\frac{u-t}{\tau_s}\right)du - \left(\frac{1}{\tau_l} - \frac{1}{R_s C_p}\right)\int_0^t E_p(u)\exp\left(\frac{u-t}{\tau_l}\right)du\right]$$

$$\frac{1}{\tau_s} = \frac{1}{2}\left[\frac{1}{R_p C_m} + \frac{1}{\tau_p} + \frac{1}{\tau_m} + \sqrt{\left(\frac{1}{R_p C_m} + \frac{1}{\tau_p} + \frac{1}{\tau_m}\right)^2 - 4\left(\frac{1}{R_p C_m R_s C_p} + \frac{1}{\tau_p \tau_m}\right)}\right]$$

$$\frac{1}{\tau_l} = \frac{1}{2}\left[\frac{1}{R_p C_m} + \frac{1}{\tau_p} + \frac{1}{\tau_m} - \sqrt{\left(\frac{1}{R_p C_m} + \frac{1}{\tau_p} + \frac{1}{\tau_m}\right)^2 - 4\left(\frac{1}{R_p C_m R_s C_p} + \frac{1}{\tau_p \tau_m}\right)}\right]$$

$$\frac{1}{\tau_p} = \frac{1}{R_p C_p} + \frac{1}{R_s C_p} \qquad \frac{1}{\tau_m} = \frac{1}{R_e C_m} + \frac{1}{R_m C_m}$$

FIG. 1. The equivalent circuit for both pulsed light and continuous light responses. $E_p(t)$ is the photoemf. R_m and C_m are the ordinary membrane resistance and capacitance, respectively. R_p and C_p are the chemical resistance and capacitance, respectively. R_s is the transmembrane resistance. R_e is the access impedance of electrodes and electrolyte solutions. I(t) is the feedback photocurrent. The negative feedback amplifier maintains points A (reductant side) and B (oxidant side) at equipotential. For a finite and nonzero R_e, the photocurrent contains two convolution terms of the photoemf with two time constants τ_s and τ_l ($\tau_s < \tau_l$). The variable of integration, u, has the dimension of time.

in the pigment channel. In the presence of the access impedance R_e, the equivalent circuit without a series capacitance C_p cannot allow any photoelectrical relaxation faster than R_eC_m (about 40 μsec) to be observed. This is contrary to the observation of the relaxation times of 5 μsec, and it is therefore mandatory to introduce the chemical capacitance C_p. The biphasic waveform of the photocurrent suggests the presence of ac-coupling of the photoemf signal. That is, there is a capacitance C_p in series with the photoemf $E_p(t)$. A transmembrane resistance R_s ($\gg R_p$) is incorporated to include the previous model (Fig. 1*A* of ref. 5) as a limiting case in the steady state. The result of mathematical analysis of the equivalent circuit is shown in Fig. 1. Notice that the presence of the access impedance R_e prevents the feedback amplifier from clamping the voltage across the membrane at the membrane–water interfaces and brings about interaction among various branches of the network and mixing of various RC time constants. This interaction is the basis of our tunable voltage clamp method. Inserting a delta function, $\delta(t)$, for the time course of $E_p(t)$ results in an expression of photocurrent consisting of two exponential terms of opposite polarity (with time constants τ_s and τ_l); the amplitudes are proportional to the reciprocal of their respective time constants, consistent with observation. In the pair of expressions for $1/\tau_s$ and $1/\tau_l$ (Fig. 1), only R_p and C_p are unknown. It can be shown that a unique and explicit solution is available for R_p and C_p (18), and R_p and C_p are thus completely determined by experimental measurements. The input data for the computed response are listed in the *legend* of Fig. 3. The model correctly predicts the opposite signs of the two relaxation components and the numerical ratio of their amplitudes.

The above model is oversimplified, because it neglects the dark reactions (5) and the fact that we illuminate specifically only a fraction of the bimolecular region of the total membrane. Further generalization leads to an equivalent circuit with separate channels for the illuminated zone and for the

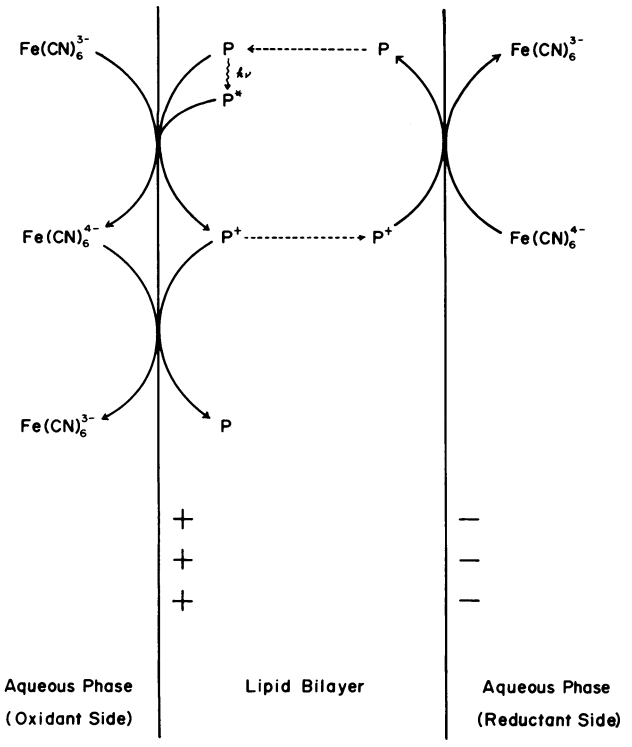


FIG. 2. The photochemical reaction cycle across the membrane-water interfaces. The ground state magnesium porphyrin P and the electron acceptor $Fe(CN)_6^{3-}$ undergo reversible one-electron oxidation-reduction to form the stable monocation P^+ and $Fe(CN)_6^{4-}$ (15). Oxidation is much enhanced in the excited state P* (16, 17). The pigment molecules P, P*, and P^+, are confined to the membrane. The electron acceptor and the donor are located in the aqueous phases. Electron transfer is thus forced to occur only across the membrane–water interfaces. The pigment molecules, both charged and uncharged, are mobile inside the lipid bilayer, and the redox reactions at both interfaces are coupled by diffusion of the pigment molecules. The plus and the minus signs at the lower part of the diagram indicate the charges on the chemical capacitance. See *text* for explanation.

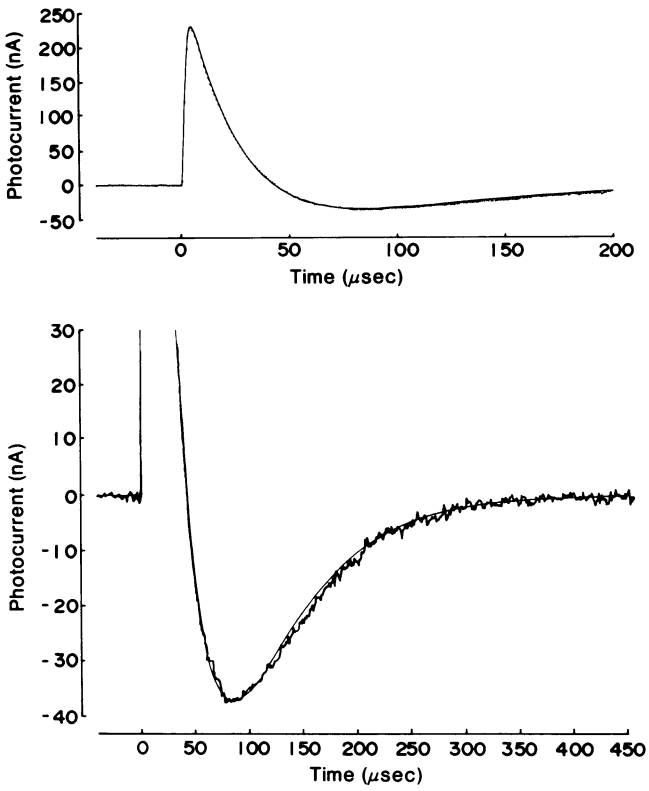


FIG. 3. Comparison of measured (dotted or noisy curve) and computed (smooth curve) photoresponses to a dye laser pulse. The measured curve is taken under the condition of voltage clamp at the electrode input (points *A* and *B* in Fig. 1) at 0 mV, from the lipid bilayer which contains magnesium mesoporphyrin IX di-*n*-amyl ester and separates two aqueous phases with 20 mM potassium ferricyanide and 0.5 mM potassium ferrocyanide on the oxidant side, and 20 mM potassium ferrocyanide on the reductant side. Both aqueous phases also contain 1 M NaCl and 10 mM phosphate buffer at pH 7.2. The temperature is held constant at 26°C. The exciting laser beam is focused to illuminate 19% of the thin bilayer of area 1.7 mm². The instrumental time constant is 1.5 μsec. The average of 16 measurements from the same membrane is obtained with a signal averager. The same averaged record is displayed in two different scales to show the two components of the relaxation of the photocurrent. The input data for the computation are all obtained from experimental measurements: $\tau_s = 29$ μsec, $\tau_l = 63$ μsec, $R_m = 1.5 \times 10^9$ Ω, $C_m = 8.2$ nf, $R_e = 5.1$ kΩ, $R_s = 10^9$ Ω, and $E_p(t)$ is taken to have the time course of an equilateral triangular pulse with a half-width of 0.3 μsec, the same as the exciting light pulse. The low-pass filtering effect of the feedback RC loop of time constant 1.5 μsec is also taken into account. The computed responses are normalized with respect to the peaks, with experimental peak amplitude ratio of 6.2. The calculated parameters are: $\tau_p = 44$ μsec, $R_p = 33$ kΩ, $C_p = 1.3$ nf, and the theoretical peak amplitude ratio of 6.6. The values of all RC parameters refer to the entire area of the thin bilayer. We note that the access impedance is actually complex because of the presence of electrode capacitance. However, for times shorter than the shortest relaxation time of the calomel electrodes (about 5 msec), the access impedance can be replaced by an effective resistance R_e. It increases only 1.6% over the time range shown.

dark zone. If we define the RC parameters for the entire area of the thin bilayer and assume that the resistances are inversely proportional to the area and the capacitances are directly proportional to the area, the results are identical to those given in Fig. 1 except with two modifications (18). An additional term, which represents the constant dark current, appears in the expression for I(t). An attenuation factor ϕ, which is the fraction of the area illuminated, appears in each convolution term.

We shall now interpret the equivalent circuit in molecular terms. The transmembrane resistance R_s is interpreted as the resistance the pigment molecules encounter when they cross the hydrocarbon core of the bilayer. Experiments show that the chemical conductance $1/R_p$ varies approximately linearly with ferrocyanide concentration in the oxidant side. Therefore, R_p is located at the membrane–water interface of the oxidant side and is the resistance to reverse electron flow across the interface. In fact, $1/\tau_p$ ($\simeq 1/R_pC_p$) varies linearly with ferrocyanide concentration in the oxidant side. We interpret $1/\tau_p$ as the pseudo-first-order rate constant of the reaction of P^+ in the bilayer with ferrocyanide in the adjacent (oxidant) aqueous phase, and the slope, $(3.5 \pm 0.4) \times 10^7$ M^{-1} sec^{-1}, of the plot as the second-order rate constant

for this reaction. In agreement with this interpretation, deviations toward second-order kinetics are seen at very low ferrocyanide concentration.

Because of the limited range of electron transfer (16, 17), only those pigment molecules close to the oxidant interface can react to form P^+. The monocation P^+ can be regarded as a fixed space charge in the microsecond time scale. Because diffusion of P^+ across the membrane is slow (as reflected by the large value of R_s), most P^+ reacts with ferrocyanide at the same interface where it was formed. This process is tantamount to charging and discharging of a capacitance (the chemical capacitance C_p). The polarity of the chemical capacitance when it is charged is such that the oxidant side is positively charged and the reductant side is negatively charged (Fig. 2). That is, it is opposite to the polarity of the photoemf. Although the charges on the chemical capacitance polarize nearly the same dielectric as do the charges on the ordinary membrane capacitance, they are distinct physical entities. Specifically, one "plate" of the chemical capacitance is charged by P^+, the other "plate" at the reductant interface is charged by inorganic ions in the aqueous phase. The charging and discharging currents must be relayed through the specific redox reaction at the interface. A theoretical calculation (F. T. Hong, unpublished) which is based on the photochemical scheme in Fig. 2 and the Gouy-Chapman theory (19) confirms this interpretation of the chemical capacitance.

The photoemf $E_p(t)$ in the present equivalent circuit is a formal representation of the photoreactions. We assign no value to this photoemf because the equivalent circuit makes no distinction between the forward charging and the reverse discharging resistance. We believe these may be very different and are in the process of determining them separately. The time course of the photoemf $E_p(t)$ is taken to be the same as that of the exciting light pulse. The success of the computation is a reassurance that the photoemf does not last appreciably longer than the exciting light pulse at the present time resolution of 0.2 μsec. This interpretation is consistent with the following facts: the electron transfer from the excited state is extremely fast; the singlet state of magnesium porphyrin lives about 1 nsec; and the triplet state lives less than 100 nsec under aerobic conditions. It is the photoemf that charges the capacitances. The relaxation of the electrical response is not due to a decay of the photoemf. The relaxation is passive and is determined by the RC arrangements in the system.

The equivalent circuit predicts that excitation with a rectangular pulse of light much longer than the intrinsic relaxation times would cause complete separation of the positive and negative responses at the "on" and the "off" times, respectively. We do not have available long light pulses of sufficiently fast risetime. However, with the use of a 50-μsec argon ion laser pulse and the assumption that the photoemf again follows the light pulse, the calculated response predicts the decrease of the photocurrent before the light pulse decays, consistent with our observations.

The above interpretation explains both the pulsed light and the continuous light experiments from a unified point of view. In the response to a short pulse of light, P^+ forms and vanishes at the same interface, and the photocurrent flows predominantly through the chemical capacitance C_p. In the response to continuous light, a steady state is set up and concentration gradients of the pigment molecules, both charged and uncharged, arise. The redox reactions at both interfaces are coupled by diffusion of the mobile pigment molecules, and the photocurrent flows predominantly through the transmembrane resistance R_s. Since R_p and C_p can be made to depend on the redox composition of the oxidant side only, it is obvious why the pulsed light response is specific to reactions at a single interface, while the continuous light response depends on reactions at both interfaces (14). It is also clear why the pulsed response requiring C_p is specific to the bimolecular region of the membrane: the annular region is far too thick (14).

A fascinating aspect on our tunable voltage clamp method has to be pointed out. Strictly speaking, this method can only be regarded as a nonideal voltage clamp method because of the presence of the access impedance. The maximal deviation of the clamping voltage is small, being only 1.1 mV in the example shown in Fig. 3. Nevertheless, the access impedance has such a profound effect on the time course of the photocurrent that actually more information is available from the measurement. Comparison with the ideal voltage clamp method (i.e., $R_e = 0$) shows that the latter provides only a single time constant τ_p ($\simeq R_pC_p$) (18). Although, in principle, the open circuit voltage measurement (i.e., $R_e = \infty$) provides two time constants, the two components differ by five orders of magnitude both in amplitudes and in time constants and thus the determination of R_p and C_p separately is difficult (18). Usually, the larger time constant has its largest contribution from the non-informative membrane RC time constant (R_mC_m), as is observed (8, 10, 12). The ability to vary R_e in our tunable voltage clamp method permits "tuning" the relaxation times into ranges convenient for observation. The observed relaxation times are shortened and the signal gain increases when R_e is reduced. These experimental results are completely consistent with the present equivalent circuit. The presence of the access impedance is therefore an asset rather than a nuisance.

Finally, we point out the implication of the present model to studies of the light-induced electric field across the chloroplast membranes (20). Our results clearly show that even for the simplest possible photoreaction across an interface, a single capacitance (that of the membrane itself) is insufficient to describe the system. We predict that in a complex system, such as the photosynthetic system in green plants, each and every interfacial reaction will have a capacitance associated with it. The emf's generated by the photoreactions are specific to each capacitance. The overall electrical response to a pulse of light will decay with more than one time constant, as is observed. It is possible that the application of our method of analysis to the early receptor potential in the retina (21) might reveal the presence of a similar capacitance. Thus, the chemical capacitance arising from photo-generated charged pigment molecules in membranes may be of fairly general occurrence.

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1. Tien, H. T. (1968) *Nature* **219**, 272–274.
2. Tien, H. T. (1968) *J. Phys. Chem.* **72**, 4512–4519.
3. Hesketh, T. R. (1969) *Nature* **224**, 1026–1028.
4. Ilani, A. & Berns, D. S. (1972) *J. Membrane Biol.* **8**, 333–356.
5. Hong, F. T. & Mauzerall, D. (1972) *Biochim. Biophys. Acta* **275**, 479–484.

6. Huebner, J. S. & Tien, H. T. (1972) *Biochim. Biophys. Acta* **256**, 300–306.
7. Kobamoto, N. & Tien, H. T. (1972) *Biochim. Biophys. Acta* **266**, 56–66.
8. Ullrich, H.-M. & Kuhn, H. (1972) *Biochim. Biophys. Acta* **266**, 584–596.
9. Tien, H. T. (1972) *Photochem. Photobiol.* **16**, 271–290.
10. Trissl, H.-W. & Laüger, P. (1972) *Biochim. Biophys. Acta* **282**, 40–54.
11. Tien, H. T. & Huebner, J. S. (1973) *J. Membrane Biol.* **11**, 57–74.
12. Schadt, M. (1973) *Biochim. Biophys. Acta* **323**, 351–366.
13. Moore, J. W. & Cole, K. S. (1963) in *Physical Techniques in Biological Research*, ed. Nastuk, W. L. (Academic Press, New York & London), Vol. VI, Part B, pp. 263–321.
14. Hong, F. T. & Mauzerall, D. (1972) *Nature New Biol.* **240**, 154–155.
15. Fuhrhop, J.-H. & Mauzerall, D. (1969) *J. Amer. Chem. Soc.* **91**, 4174–4181.
16. Mauzerall, D. (1973) *Ann. N.Y. Acad. Sci.* **206**, 483–494.
17. Carapellucci, P. & Mauzerall, D. (1974) *Ann. N.Y. Acad. Sci.*, in press.
18. Hong, F. T. (1973) Ph.D. Dissertation (The Rockefeller University, New York, New York).
19. Verwey, E. J. W. & Overbeek, J. Th. G. (1948) in *Theory of the Stability of Lyophobic Colloids* (Elsevier, Amsterdam & New York).
20. Witt, H. T. (1971) *Quart. Rev. Biophys.* **4**, 365–477.
21. Murakami, M. & Pak, W. L. (1970) *Vision Res.* **10**, 965–975.